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Original Article

Relative abundance and diversity of man-biting mosquito species before and after indoor residual spraying programme in Awka and Environs, Anambra State, Nigeria

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ABSTRACT

The availability of mosquito vectors is an important epidemiological factor in the transmission of mosquito borne diseases. The abundance of man-biting mosquito species was studied before and after indoor residual spraying (IRS) in three communities in Awka North and South Local Government Areas, Anambra State, Nigeria, between April and December 2013. Indoor biting and resting adult mosquitoes were collected using pyrethrum knockdown collection (PKC) method. Outdoor biting adults were collected using human bait collection (HBC) method. Larvae were collected by scooping. Chi square x^2, ANOVA and Simpson's diversity index were used for data analysis. A total of 12,948 mosquitoes were collected. Larval collection was highest 9,871 (76.24%), indoor biting adults were 2,552 (19.71%) while the least was outdoor biting adults 525 (4.05%). The pre-residual spray collection of the mosquitoes 8,507 (65.70%) was almost twice higher than the post-residual spraying collections 4,441 (34.30%), and there was a significant difference ($p = 0.000$, $p < 0.05$). Mosquito species collected were *Culex quinquefasciatus* 1,437 (46.70%), *Anopheles gambiae* 1054 (34.25%), *An. funestus* 61 (1.98%), *Aedes albopictus* 257 (8.35%), *Ae. aegypti* 250 (8.12%) and *Ae. bromeliae* 18 (0.58%). *Culex quinquefasciatus* was the most abundant. The Simpson's index of mosquitos' diversity was higher during post-IRS (0.779) than pre-IRS (0.614). Indoor residual spraying was found to be a very effective mosquito vector control strategy. Occasional implementation of IRS and engagement of communities by government is recommended for efficient vector control.

KEYWORDS: mosquitoes, vectors, abundance, diversity, IRS, Awka.

INTRODUCTION

Mosquitoes are vectors of different parasitic diseases of man [1-3]. Vector control remains an important component in controlling these diseases [4]. Vector control protects people by preventing, reducing or interrupting the transmission of vector borne diseases such as malaria [5]. The different methods of vector control available include long-lasting insecticide-treated nets (LLINs) and IRS [6, 7]. House spraying remains a valuable tool in malaria control when implemented properly. Indoor residual spraying is the application of long-acting chemical insecticides on walls and roofs of houses inhabited by humans and shelters of domestic animal in a given area, in order to kill malaria vectors and other mosquito species that land and rest on these surfaces [8]. In some situations, IRS can lead to the elimination of locally important malaria vectors [9].

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Indoor residual spray has been used widely in many areas of the world, especially in Asia, Latin America and Southern Africa. Indoor residual spraying with DDT and other insecticides has been one of the main interventions leading to the elimination of malaria in about half of the world's regions, for example in most of Southern Europe, North America, Japan, Central Asia and Latin America [10, 11].

In Nigeria, evidence has shown that IRS at 85% coverage of target risk populations, and target structures remains the most efficacious and effective intervention that reduces malaria transmission rapidly at an affordable cost [7]. The National Malaria Control Programme of Nigeria and its partners have conducted small scale pilots IRS projects in different parts of the country with promising outcome [12]. This study was to evaluate the effectiveness of IRS program in controlling mosquito vectors of public health diseases in the study communities. The specific objectives were to determine mosquito species density and their relative abundance in the selected communities before and after indoor residual spraying.

MATERIALS AND METHODS

Study area

The study was conducted in three communities; Amansea and Ebenebe in Awka North and Awka metropolis in Awka South Local Government Area. Awka North and Awka South Local Government Areas are within the capital territories of Anambra State of Nigeria. The geographical coordinates are 6º, 9' and 6º, 24' North latitude and 6º, 58' and 7º, 10' East longitude. They are located in the tropical rainforest zone, although the vegetation can be described as derived Guinea savannah. The area has two marked seasons - the dry and wet seasons. It has a relative humidity of 70% reaching 80% during rainy season and an annual rainfall of about 2000-3000 mm [13]. The daily temperature ranges from 26-35 ºC during the dry season (November to February) and from 22-30 ºC during wet season (March to October). Awka (urban) shares a boundary with Amansea (sub-urban) while Amansea shares boundary with Ebenebe (rural). Amansea has a large population of Hausa and Fulani herdsmen and traders and a large cattle market while Awka is the capital city of Anambra State, with various government institutions and establishments. Ebenebe is a rural area with a lot of farm lands and low population densities. The population of Awka North is 112,192 while Awka South is 189,654 [14]. The people are ethnically Igbos. The topography of the area makes it is prone to erosion leading to formation of potholes and gullies, which may serve as possible sites for mosquito breeding.

Community visitation and mobilization

Permission to carry out the study in the communities was obtained from their opinion leaders. Informed consent of the heads of households whose compounds were used for the study was obtained through proper explanation of the purpose of the study. The general community was mobilized through announcements in schools, churches, markets, town meetings and town criers. All volunteer mosquito collectors were properly informed of the nature of the study. They were given yellow fever vaccines 12 days before the commencement of the study.

Study design and sampling techniques

A cross sectional survey of the community was used to determine population of mosquitoes before and after indoor residual spraying. Bi-weekly collections of mosquitoes from the selected communities were done to estimate their relative abundance. Indoor biting adult mosquitoes were collected using PKC method, outdoor biting adult mosquitoes were collected using HBC method while collection of immature stages of the mosquitoes were done by scooping method using ladles [15]. Random sampling technique was used to select 24 households (Eight households in each of the communities) for the study.

Collection of outdoor biting adult mosquitoes using human bait collection method

The collection of outdoor biting adult mosquitoes in the communities was carried out between 17.00 and 20.00 hours (5.00-8.00 pm local time). The landing and biting catch recommended by WHO [16] was used to collect adult mosquitoes from study areas. Four human volunteers were involved

in the collection of man-biting adult mosquitoes. Materials used were torch lights, test-tube vials, cotton wool, wrist watches for keeping time, pens and papers for recording the time of collections, and cellophane bags for collation of catches. All catches were recorded at quarter-hourly intervals [15, 17]. The four volunteers rolled up their shirt sleeves and pairs of trousers to their elbows and knees respectively, put off their shoes and sandals and each sat on a low stool, a short distance away from each other. They searched meticulously over their bodies for the arrival and alighting of any mosquito. Mosquitoes alighting on their body to suck blood were collected with a test tube vial, stoppered with a ball of cotton wool, the time of collection recorded and then kept separate [18]. At the end of each day's collection, the collections were sorted into quarter-hourly collections and placed in separate cellophane bags.

Collection of indoor biting and resting adult mosquitoes using pyrethrum knockdown collection method

Indoor biting and resting adult mosquitoes were collected using PKC between the hours of 6.00 am and 9.00 am [16]. Large white sheets were laid wall to wall on floors of the rooms and all doors and windows were shut. A pyrethriod-based insecticide aerosol (Baygon) was sprayed inside the rooms. For houses without ceilings, the house eaves which may serve as possible escape route of mosquitoes were also sprayed from outside. After 20 minutes, the spread sheets were systematically folded and taken outside where the mosquitoes were collected using forceps, into a wet Petri dish overlayed with filter paper placed over dampened cotton wool.

Collection of mosquito larvae

Larval mosquitoes were sampled from water in discarded or used tyres, ground pools such as rainwater collections on the roads, pools of water around public taps, potholes, gutters, and ground water pools around houses and domestic reservoirs such as earthen pots, water drums, plastic buckets, cans, and tins of assorted types. Ladles were used for collection of larvae in ground pools, earthen pots and discarded tyres [15, 16]. All collected larvae were stored with little water from the breeding ground in large labeled specimen bottles (jam jars) covered with mosquito nettings to provide ventilation. The larvae were sent to the National Arbovirus and Vectors Research Centre Laboratory Enugu for rearing to adult and proper identification.

Identification of collected mosquitoes

The identification of collected mosquitoes was done at the National Arbovirus and Vector Research Centre Laboratory, Enugu. The mosquitoes were identified using the gross morphology of the species, including the external morphology of the palps, antenna, proboscis, patches of pale and black scales on the wings and legs and the terminal abdominal segments [15, 19].

Data analysis

Chi square x^2 and analysis of variance (ANOVA) was used to compare statistical means at 0.05 confidence. Simpson's diversity index was used to calculate the density and abundance of mosquitoes before and after indoor residual spraying.

RESULTS

A total of 12,948 mosquitoes were collected in the study. Larval mosquitoes was highest (9,871 (76.24%)), followed by indoor biting and resting adult mosquitoes (2,552 (19.71%)) while outdoor biting adult mosquitoes were the least 525 (4.05%). The pre-IRS collection of the mosquitoes was almost twice (8,507 (65.70%)), higher than the post-IRS collections (4,441 (34.30%)). Overall, larval collection has the highest Simpson's index of mosquito diversity (0.778) while indoor biting and resting mosquito collection has the least (0.503). Human bait collection index was 0.532. A total of 3,077 adult mosquitoes were collected from the three communities. Of this number, 855 (27.79%) were from Ebenebe, 947 (30.78%) from Amansea and 1,275 (41.44%) from Awka (Table 1). The distribution of six mosquito species collected is shown in Table 1. *Culex quinquefasciatus* was the most abundant (1,437 (46.70%)) while *Ae. bromeliae* was the least (18 (0.58%)). The pre-IRS population of the mosquitoes were significantly higher than their post-IRS population ($p < 0.05$).

The pre-IRS Simpson's index of mosquito diversity was highest (0.620) in Ebenebe while

Table 1. Pre and post distribution of adult mosquitoes in the sampled communities.

Period of collection	Species of mosquito	Communities			Total
		Ebenebe	Amansea	Awka	
Pre-IRS collection	*Culex quniquefasciatus*	301 (22.07%)	444 (32.56%)	619 (45.38%)	1364 (44.33%)
	Anopheles gambiae	336 (33.91%)	311 (31.38%)	344 (34.71%)	991 (32.21%)
	Anopheles funestus	8 (21.05%)	11 (28.94%)	19 (50.00%)	38 (1.23%)
	Aedes albopictus	40 (23.39%)	40 (23.39%)	91 (53.22%)	171 (5.56%)
	Aedes aegypti	41 (24.26%)	52 (30.77%)	73 (43.20%)	169 (5.50%)
	Aedes bromeliae	12 (100%)	0 (0.00%)	0 (0.00%)	12 (0.39%)
	Sub total	741 (26.99%)	858 (31.26%)	1146 (41.75%)	2745 (89.21%)
Post-IRS collection	*Culex quniquefasciatus*	26 (35.62%)	13 (17.81%)	34 (46.58%)	73 (21.99%)
	Anopheles gambiae	19 (30.16%)	33 (52.38%)	11 (17.46%)	63 (18.98%)
	Anopheles funestus	3 (13.04%)	6 (26.09%)	14 (60.87%)	18 (6.93%)
	Aedes albopictus	37 (43.02%)	18 (20.93%)	31 (36.05%)	86 (25.90%)
	Aedes aegypti	23 (28.40%)	19 (23.46%)	39 (48.15%)	81 (24.40%)
	Aedes bromeliae	6 (100%)	0 (0.00%)	0 (0.00%)	6 (1.81%)
	Sub total	114 (34.34%)	89 (26.81%)	129 (38.86%)	332 (10.79%)
Total Pearson Chi-Square (109.755)		855 27.79%	947 30.78%	1275 41.44%	3077 100.0%

p = 0.000.

the least (0.595) was observed in Amansea (Table 2). At post-IRS, the Simpson's index of mosquito diversity was also highest (0.771) in Ebenebe. The least (0.750) was observed in Amansea. The Simpson's index of mosquitos' diversity was higher during post-IRS (0.779) than pre-IRS (0.614).

Of the 9,871 mosquito larvae collected, 5,762 (58.37%) were collected before IRS while 4,109 (41.65%) were collected after IRS (Table 3). The pre-spray larval collection was significantly higher than the post spray collections ($p < 0.05$). The larvae collection was 3,752 (38.01%) from Ebenebe, 2,704 (27.39%) from Amansea and 3,415 (34.60%) from Awka. The mosquito species collected and their distribution are shown in Table 3.

The pre-IRS Simpson's index of mosquito larvae diversity was highest (0.797) in Ebenebe, but least (0.728) in Awka (Table 4). At post-IRS, the Simpson's index of mosquito larvae diversity was also highest (0.782) in Ebenebe and least (0.720) in Awka. The Simpson's index of diversity of mosquito larvae was higher during pre-IRS (0.778) than post-IRS (0.766).

During HBC, *Ae. albopictus* and *Ae. aegypti* were collected (Table 5). The mean abundance of *Ae. albopictus* before IRS was higher (60.00) than after IRS (8.56). The mean abundance of *Ae. aegypti* before IRS was also higher (55.00) than after IRS (9.56). Before IRS, the relative abundance of *Ae. albopictus* (35.43) was higher than that of *Ae. aegypti* (32.48). Also after IRS, the relative abundance of *Ae. albopictus* (15.16) was lower than that of *Ae. aegypti* (16.93). The abundance of the two species of mosquitoes before and after IRS showed a significant difference ($p < 0.05$).

In the three communities, the mean abundance of mosquitoes was higher before IRS than after IRS

Table 2. Diversity of adult mosquitoes before and after IRS in different locations.

IRS	Communities	Simpson's index
Pre-	Ebenebe	0.620
	Amansea	0.595
	Awka	0.608
	Total	0.614
Post-	Ebenebe	0.771
	Amansea	0.750
	Awka	0.762
	Total	0.779

Table 3. Pre and post distribution of mosquito larvae in the sampled communities.

Period of collection	Species of mosquito	Communities			Total
		Ebenebe	Amansea	Awka	
Pre-IRS collection	*Culex quniquefasciatus*	526 (44.88%)	329 (28.07%)	317 (27.05%)	1172 (11.87%)
	Anopheles gambiae	327 (32.96%)	332 (33.47%)	333 (33.57%)	992 (10.05%)
	Anopheles funestus	15 (13.51%)	27 (24.32%)	69 (62.16%)	111 (1.12%)
	Aedes bopictus	417 (27.10%)	327 (21.25%)	795 (51.66%)	1539 (15.59%)
	Aedes aegypti	473 (29.90%)	431 (27.24%)	678 (42.86%)	2827 (28.76%)
	Aedes bromeliae	366 (100%)	0 (0.00%)	0 (0.00%)	366 (3.71%)
	Sub total	2124 (36.86%)	1446 (25.10%)	2192 (38.04%)	5762 (58.37%)
Post-IRS collection	*Culex quniquefasciatus*	305 (30.32%)	367 (36.48%)	334 (33.20%)	1006 (10.19%)
	Anopheles gambiae	155 (33.41%)	169 (36.42%)	140 (30.17%)	464 (4.70%)
	Anopheles funestus	11 (20.00%)	17 (30.90%)	27 (49.09%)	55 (0.56%)
	Aedes bopictus	391 (37.45%)	405 (38.79%)	248 (23.75%)	1044 (10.58%)
	Aedes aegypti	471 (37.83%)	300 (24.10%)	474 (38.07%)	1245 (12.61%)
	Aedes bromeliae	295 (100%)	0 (0.00%)	0 (0.00%)	295 (2.99%)
	Sub total	1628 (39.62%)	1258 (30.62%)	1223 (29.76%)	4109 (41.63%)
Total Pearson Chi-Square (109.755)		3752 (38.01%)	2704 (27.40%)	3415 (34.60%)	9871 (100%)

p = 0.000.

(Table 6). Before IRS, the relative abundance of mosquitoes was highest in Awka (35.43) while the least was observed in Ebenebe (13.58). After IRS, the relative abundance of mosquitoes was highest in Ebenebe (14.46) while the least was in Amansea (6.50). The abundance of mosquitoes before and after IRS showed a significant difference ($p < 0.05$).

Table 4. Diversity of mosquito larvae before and after IRS in different communities.

IRS	Communities	Simpson's index
Pre-	Ebenebe	0.797
	Amansea	0.755
	Awka	0.728
	Total	0.778
Post-	Ebenebe	0.782
	Amansea	0.736
	Awka	0.720
	Total	0.766

Table 5. Abundance of mosquito species before and after indoor residual spraying, using human bait collection method, in the sampled communities.

Treatment	Species of mosquitoes	Mean abundance*	Relative abundance
Pre-IRS	*Aedes albopictus*	60.00[a]	35.43
	Aedes aegypti	55.00[a]	32.48
Post-IRS	*Aedes albopictus*	8.56[b]	15.16
	Aedes aegypti	9.56[b]	16.93
F-ratio	Spp. of mosquitoes	0.333	
	Treatment	195.49**	

*Column followed by the same superscript is not significantly different. ** $p < 0.05$.

Table 6. Abundance of mosquito species before and after indoor residual spraying using human bait collection method in the sampled communities.

Treatment	Communities	Mean abundance of mosquitoes*	Relative abundance of mosquitoes
Pre-IRS	Ebenebe	23.00[c]	13.58
	Amansea	32.00[b]	18.90
	Awka	60.00[a]	35.43
Post-IRS	Ebenebe	8.44[d]	14.96
	Amansea	3.67[d]	6.50
	Awka	6.00[d]	10.63
F-ratio	Communities	799.509**	
	Treatment	236.203**	

*Column followed by the same letter is not significantly different. ** $p < 0.05$.

Culex quinquefasciatus, *An. gambiae* and *An. funestus* were captured by PKC (Table 7). The mean abundance of *C. quinquefasciatus* in the communities was highest before IRS (455.33) and least after IRS (8.11). The mean abundance of *An. gambiae* in the communities was also highest before IRS (330.33) and least after IRS (5.89). The mean abundance of *An. funestus* in the sampled communities was highest before IRS (14.33) and lowest after IRS (3.00). Before IRS, the relative abundance of *C. quinquefasciatus* was highest (53.61) while that of *An. funestus* was least (32.48). After IRS, the relative abundance of *C. quinquefasciatus* remained highest (2.86) while that of *An. funestus* was least (1.06). The abundance of species of mosquitoes before and after IRS showed a significant difference ($p < 0.05$).

Before IRS (Table 8), the relative abundance of mosquitoes was highest in Awka (36.88) and least in Ebenebe (25.99). After IRS, the relative abundance of mosquitoes was highest in Awka (3.65) but least in Amansea (1.06). The abundance

Table 7. Abundance of mosquito species in the sampled communities before and after indoor residual spraying using pyrethrum knockdown collection method.

Treatment	Mosquitoes species	Mean abundance*	Relative abundance
Pre-IRS	*Culex quinquefasciatus*	455.33[a]	53.61
	Anopheles gambiae	330.33[b]	38.89
	Anopheles funestus	14.33[c]	1.49
Post-IRS	*Culex quinquefasciatus*	8.11[c]	2.86
	Anopheles gambiae	5.89[c]	2.08
	Anopheles funestus	3.00[c]	1.06
F-ratio	Spp. of mosquitoes	1526.267**	
	Treatment	5905.74**	

*Column followed by the same letter is not significantly different. ** $p < 0.05$.

Table 8. Abundance of mosquitoes by communities before and after indoor residual spraying using pyrethrum knockdown collection method.

Treatment	Communities	Mean abundance of mosquitoes*	Relative abundance of mosquitoes
Pre-IRS	Ebenebe	220.33[c]	25.99
	Amansea	263.67[b]	31.10
	Awka	312.67[a]	36.88
Post-IRS	Ebenebe	3.67[e]	1.29
	Amansea	3.00[f]	1.06
	Awka	10.33[d]	3.65
F-ratio	Communities	0.070	
	Treatment	3000.033**	

*Column followed by the same letter is not significantly different. ** $p < 0.05$

of mosquitoes showed a significant difference between communities ($p < 0.05$).

DISCUSSION

A total of 12,948 adults and larval mosquitoes were collected during the study. Of this number, 8,507 mosquitoes were pre-IRS collections and were significantly higher than the post-IRS mosquito population, 4,441. This implies that the abundance of mosquitos in the three communities was higher before IRS than after IRS. This finding corroborates with another report [20] in Kwa-Zulu Natal, an epidemic province in South Africa and also [21] in Mozambique, Botswana, Namibia, and Zimbabwe all in Southern Africa. In 2006 and 2007, the national malaria control programme in Nigeria and its partners conducted a small scale pilot projects which showed effectiveness of IRS in controlling local malaria vectors [12]. These findings were in contrast to the experimental hut studies in Burkina Faso in which a combination of pyrethroid-treated wall linings and insecticide-treated nets (ITNs) failed to induce any increase in mortality of malaria vectors [22]. This difference can be attributed partly to the fact that the vector population in the study area is fully susceptible to the active component (Deltamethrin and lambda-cyphalothrin) in the IRS treatment and partly to the effectiveness of the insecticide used. The vectors decreased despite the abundance of rainfall which is an important factor that promotes mosquito breeding especially by providing many breeding sites and high relative humidity which prolongs the longevity of the adult mosquitoes [21, 23, 24].

Six mosquito species namely *C. quinquefasciatus*, *An. gambiae*, *An. funestus*, *Ae. albopictus*, *Ae. aegypti* and *Ae. bromeliae* were collected from the three communities studied. These observations are in tandem with another finding [17] which reported the same genera and species of mosquitoes at the development site of Nnamdi Azikiwe University, Awka. *Anopheles gambiae*, *An. funestus*, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus* have also been reported in another study in other communities near the study area [25]. Also, same mosquito genera have been observed in different parts of Nigeria [26-29]. All anophelines were collected from inside houses which could be attributed to the search for blood meal sources mainly humans sleeping indoors, and also higher indoor temperatures [30, 31].

Culex quinquefasciatus was the most abundant mosquito species during PKC. Before IRS, the relative abundance of *C. quinquefasciatus* was highest while *An. funestus* was least. Also, after IRS, the relative abundance of *C. quinquefasciatus* remained highest while *An. funestus* was least. These agree with similar observation in others studies where *C. quinquefasciatus* was the most abundant in others parts of Anambra State, Nigeria [2, 3]. The dominance of *C. quinquefasciatus* over other mosquito species could be as a result of the presence of preponderance of blocked drainages with very dirty stagnant water, and septic tanks among others which serve as their breeding sites found in the study area [25, 27].

The collection of 9,871 mosquitoes as larvae from the different breeding sites in the communities is an indication of intensive breeding of mosquitoes in the area as well as preponderance of their breeding sites. This finding corroborates with an earlier report [27] that the preponderance of mosquitoes in Awka metropolis was due to prevailing habitats in the area. The prevailing breeding habitat observed in the area during the study includes, ground pools, dirty blocked gutters, abandoned vehicle tires, discarded containers, leafs and plant axils amongst others.

CONCLUSION

This study demonstrated that mosquitoes still breeds and bites in the study areas. On the other hand, the study also revealed the effectiveness of IRS intervention in controlling the local mosquito vectors since its efficiency in reducing vector population has been proven through this study. The finding from the present studies also shows that IRS is an effective malaria vector control strategy since it also reduced the population of anopheles species. Therefore, occasional implementation of IRS and engagement of communities by government as well as monitoring and evaluation of the vector control programmes is recommended for efficient vector control.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

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